Project No.
132 Book No. TITLE New 3'-5' exo nuclease Mutant of Pf

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Purpose: Ca Previous clone (P. 129) of a 3'-5' exonuclease mutant of Thermotoga neo. polefina (Tne) proved not to produce overexpress a heat sensitive or hea polymerase activity. Here Gre DeD ~~purification~~ made a new clone of the purpose of this experiment is to screen pre + post heat kill for polymerase activity. If activity is heat thermal stable then procede w/ a PEI + $(NH_4)_2SO_4$ ppt.

3 grams of cells - resuspend in 4 ml of crack buffer - Sonicate with ~~microtip~~ micro tip ~~sonicator~~

crack buffer -
20 mM tris pH 7.5
10 mM KCl
.1 mM EDTA
5 mM Bme
5% glycerol

A_{575} = .824 before crack

A_{575} = .193 after crack 6 x 20 sec microtip @ setting 4.

70% crack -

Save 400 µl -> No heat treatment

Aliquot the rest of the cracked material to 2 ml eppendorfs
heat Kill 10 min -> temp. @ 80° - 90° c -
note: temperature rose to > 90 for maybe up to 5 min

Spin in microfuge @ 14000 g 30 minutes -

Heat treat supernatant < 90°c, > 85°c - for 5 minutes -
spin in microfuge @ 14000 x g 10 minutes -

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say for thermostable polymerase activity -

mix - TAQ premix - premade by A.G. -

add 1.1 µl / 500 µl premix
of d32D dCTP

Dilution 5/495 - $\frac{10}{90}$ - $\frac{1}{1000}$

$\frac{10}{290}$ - $\frac{1}{3000}$

1/1000 1 µl
2 µl
1/3000 1 } heat treated
2

1/1000 1
2 } before heat treatment

1/3000 1
2 } mistake made - put 78 µl of premix should have only used 48 !!

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Incubate 10' @ 74°C in a heated water block - quench w/ 10µl of .5M EDTA - Spot 30µl on GF/C 11 dia
Wash filts 1x 10% TCA 5'
3x 5% TCA 3'
2x 5 to H
dry + count in econofluor LSF

AM	CPM1	
1 1/1000	2994.00	H Kill
2	2384.00	
3 1/2000	622.00	
4	888.00	
5 1/1000	3612.00	No H Kill
6	5296.00	
7 1/2000	1234.00	
8	1662.00	
9	964.00	
10	90094.00	
11	89736.00	S.A
12	89120.00	
13	40.00	

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First approx. - looks as though not as much activity after heat & kill - need to do less dilutions to in order to ascertain what exactly is going on.

seeing host polymerase act. in No Heat Kill?

Repeat w/ 1/500 1/200 dil'ns -

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